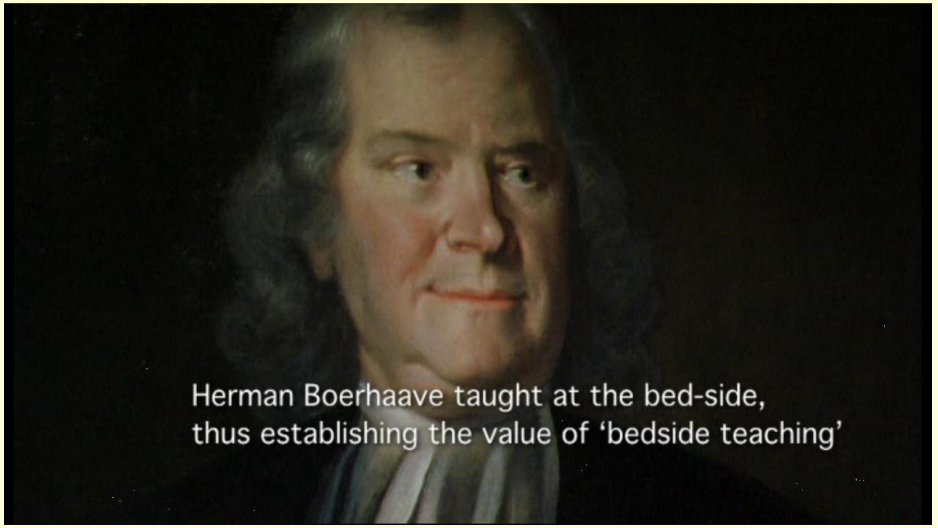
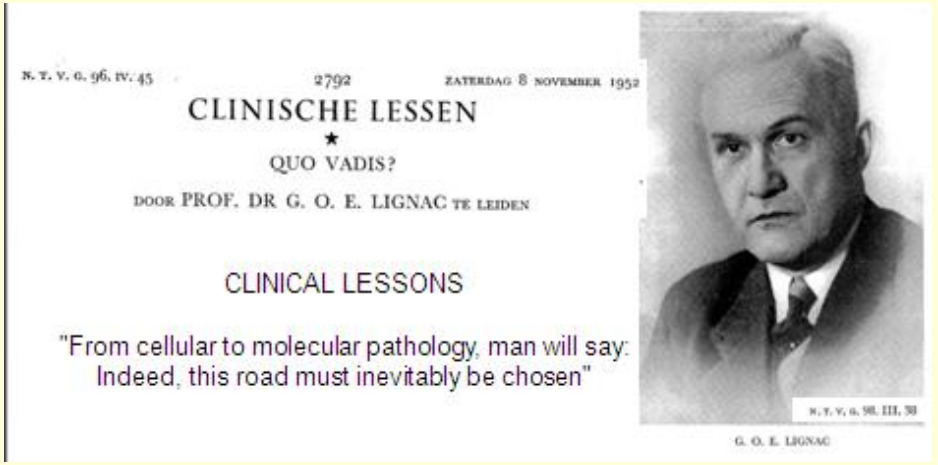
Next generation sequencing in the context of current clinical practice: Implementation and challenges





CCKL

slmp
Samenwerkende Laboratoria voor de Moleculaire Pathologie

Accreditation of molecular pathology since 2006

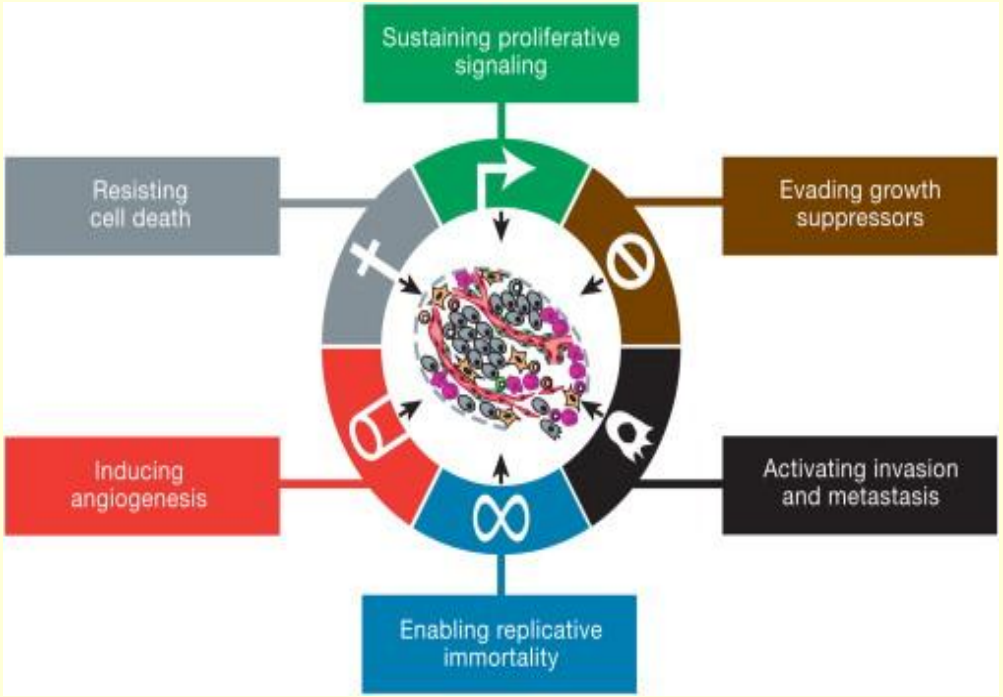
Next generation sequencing of the cancer genome in the context of current clinical practice:
Implementation and challenges

*www.slideworld.org/...aspx/**personalized-medicine***

BIOLOGY OF CANCER
Networks of signal transduction pathways in the cell.

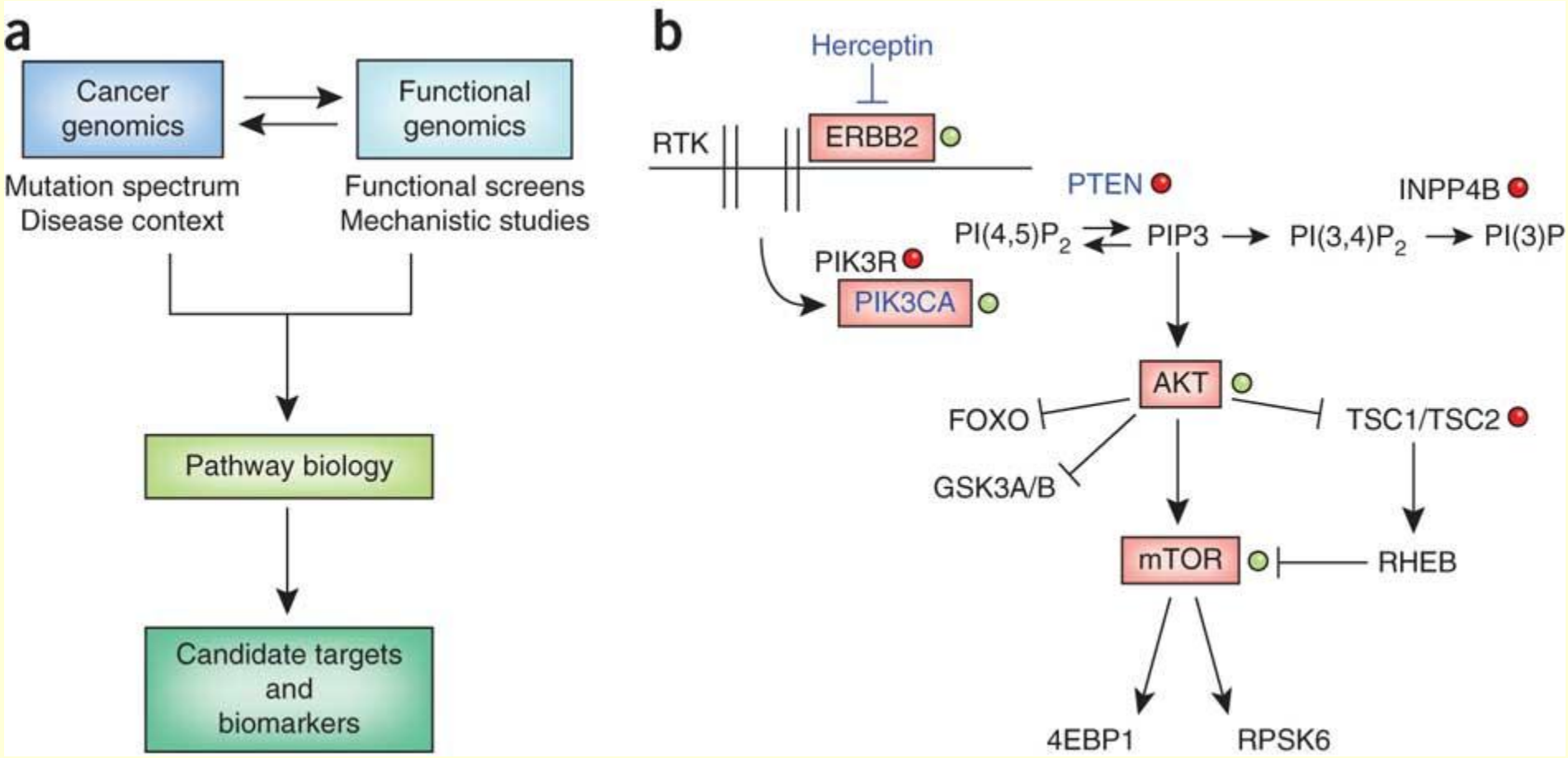
- Critical nodes (‘hubs’) in the signal transduction network are targets for oncogenic alterations

- Hanahan and Weinberg 2000/2011



Taming the dragon: genomic biomarkers to individualize the treatment of cancer

Nature Medicine 2011



Targeted Therapeutics in Cancer.

Table 1. Targeted Therapeutics in Cancer.*

Gene	Genetic Alteration	Tumor Type	Therapeutic Agent
Receptor tyrosine kinase			
EGFR	Mutation, amplification	Lung cancer, glioblastoma	Gefitinib, erlotinib
ERBB2	Amplification	Breast cancer	Lapatinib
FGFR1	Translocation	Chronic myeloid leukemia	PKC412, BIBF-1120
FGFR2	Amplification, mutation	Gastric, breast, endometrial cancer	PKC412, BIBF-1120
FGFR3	Translocation, mutation	Multiple myeloma	PKC412, BIBF-1120
PDGFRA	Mutation	Glioblastoma, gastrointestinal stromal tumor	Sunitinib, sorafenib, imatinib
PDGFRB	Translocation	Chronic myelomonocytic leukemia	Sunitinib, sorafenib, imatinib
ALK	Mutation or amplification	Lung cancer, neuroblastoma, anaplastic large-cell lymphoma	Crizotinib
c-MET	Amplification	Gefitinib-resistant non–small-cell lung cancer, gastric cancer	Crizotinib, XL184, SU11274
IGF1R	Activation by insulin-like growth factor II ligand	Colorectal, pancreatic cancer	CP-751,871, AMG479
c-KIT	Mutation	Gastrointestinal stromal tumor	Sunitinib, imatinib
FLT3	Internal tandem duplication	Acute myeloid leukemia	Lestaurtinib, XL999
RET	Mutation, translocation	Thyroid medullary carcinoma	XL184
Non–receptor tyrosine kinase			
ABL	Translocation (BCR-ABL)	Chronic myeloid leukemia	Imatinib
JAK2	Mutation (V617F), translocation	Chronic myeloid leukemia, myeloproliferative disorders	Lestaurtinib, INCB018424
SRC	Overexpression	Non–small-cell lung cancer; ovarian, breast cancer; sarcoma	KX2–391, dasatinib, AZD0530
Serine–threonine–lipid kinase			
BRAF	Mutation (V600E)	Melanoma; colon, thyroid cancer	SB-590885, PLX-4032, RAF265, XL281
Aurora A and B kinases	Overexpression	Breast, colon cancer; leukemia	MK-5108 (VX-689)
Polo-like kinases	Overexpression	Breast, lung, colon cancer; lymphoma	BI2536, GSK461364
MTOR	Increased activation	Renal-cell carcinoma	Temsirolimus (CCI-779), BEZ235
PI3K	PIK3CA mutations	Colorectal, breast, gastric cancer; glioblastoma	BEZ235
DNA damage or repair			
BRCA1 and *BRCA2*	Mutation (synthetic lethal effect)	Breast, ovarian cancer	Olaparib, MK-4827 (PARP inhibitors)

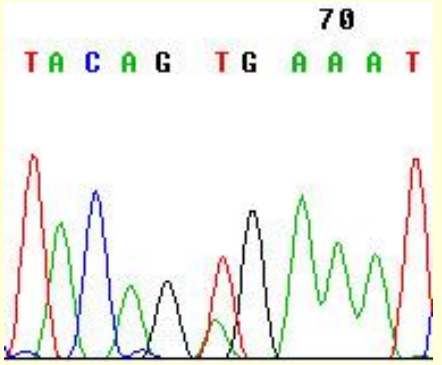
* PARP denotes poly(adenosine diphosphate–ribose) polymerase.

The NEW ENGLAND JOURNAL of MEDICINE

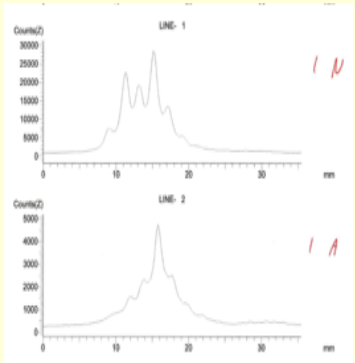
Daily practice

- Amplification
- Clonality
- Genomic alterations/CIN
- Mutation
- Methylation (CpG islands)
- MSI analysis
- Translocation
- (mi)RNA expression
- Ploidie status
- Typing

BRAF 1796T>A (V600E)

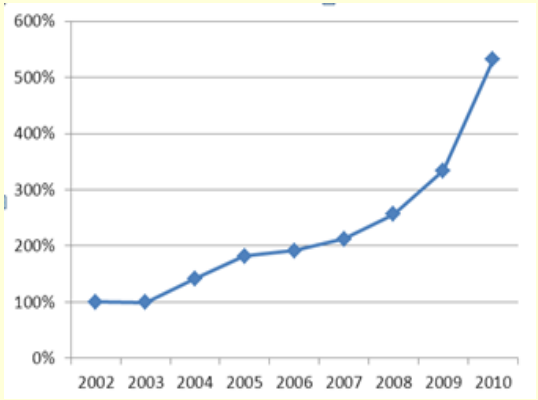


MSI Analysis



Current tools

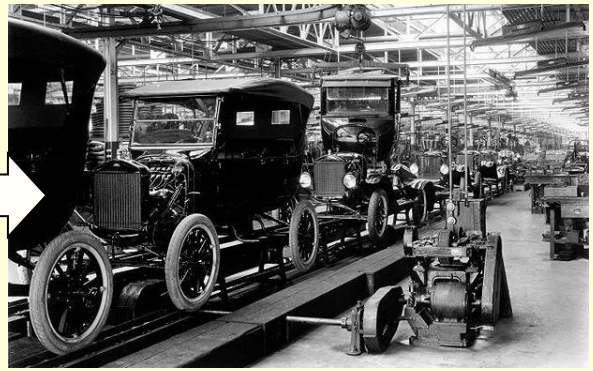
- Allele specific assays
- Classic Sanger DNA sequencing
- Flow cytometry
- Fragment analysis (typing, MSI)
- FISH, CISH/BRISH
- LIPA
- MLPA
- Melting curve analysis
- RT-PCR



- Challenges 2012-….

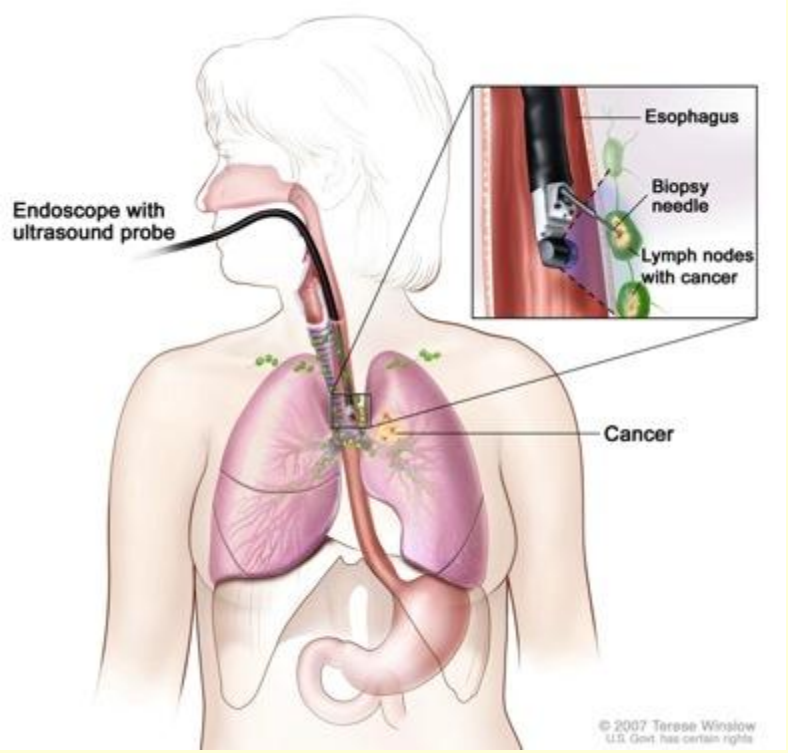
- Increasing numbers with: *Limited (pre-)operative material*
  - Early diagnosis, Neoadjuvant therapies
  - Only tissue biopsies/cores
  - Enrichment/microdissection steps
  - FNA material
- Identification of tumorheterogeneity
- Laboratory automation

Automation



Preoperative Staging: on Cytological material

- Current lung cancer staging guidelines acknowledge:
  - Endosonography with fine needle aspiration
    - Mediastinal lymph nodes
  - Minimally invasive alternative to surgical staging to detect nodal disease.
  - Minimal material

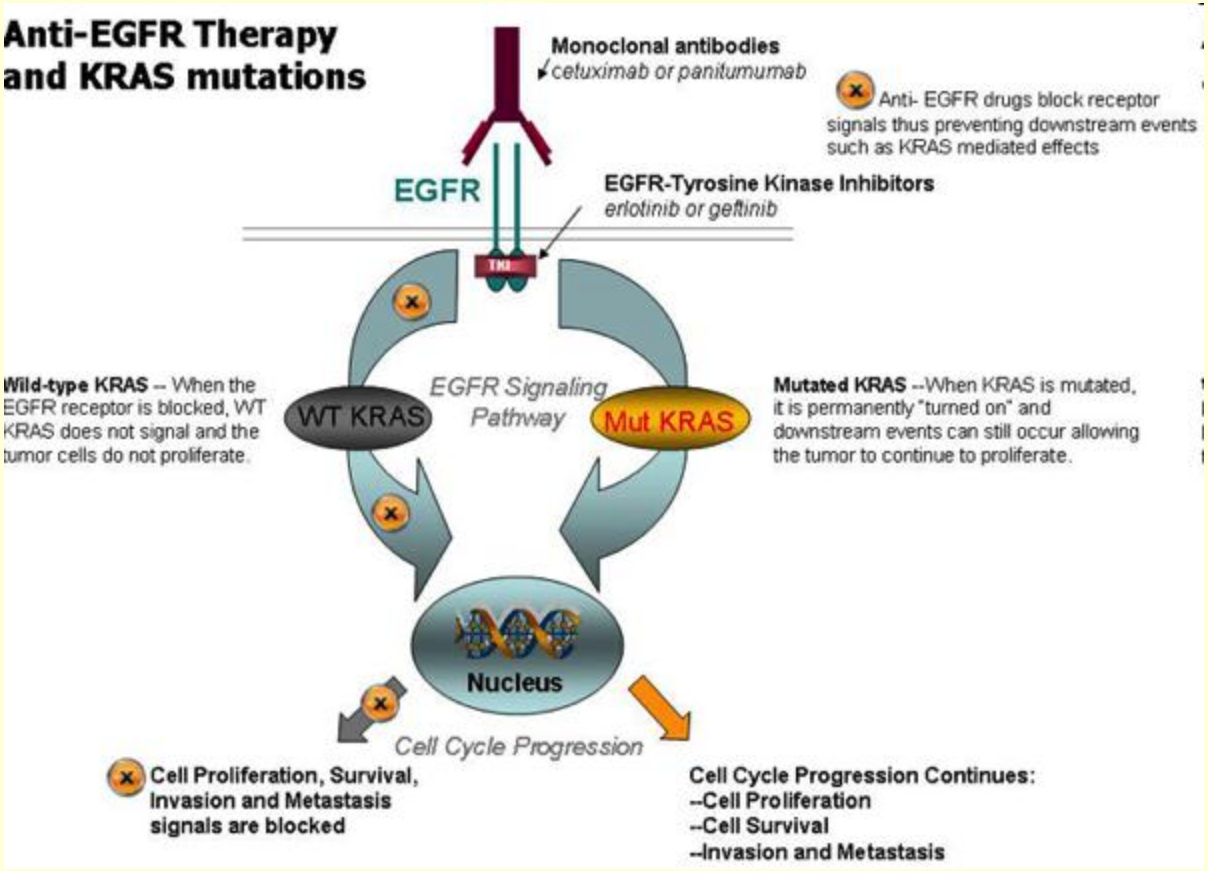


Pathological diagnosis: On Cytology from lymph nodes
Molecular Pathology on the same material: Option for Personalized medicine?

J. Annema et al JAMA. 2010;304(20):2245-2252

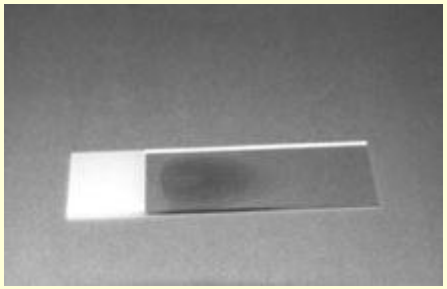
Mediastinoscopy vs Endosonography for Mediastinal Nodal Staging of Lung Cancer
A Randomized Trial

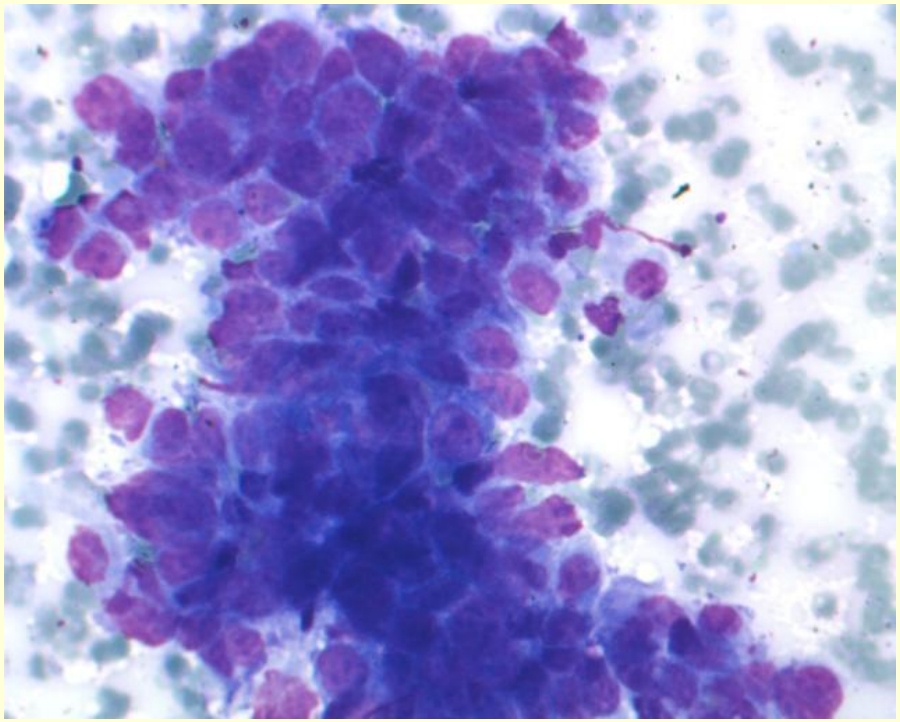
EGFR activated, KRAS wildtype



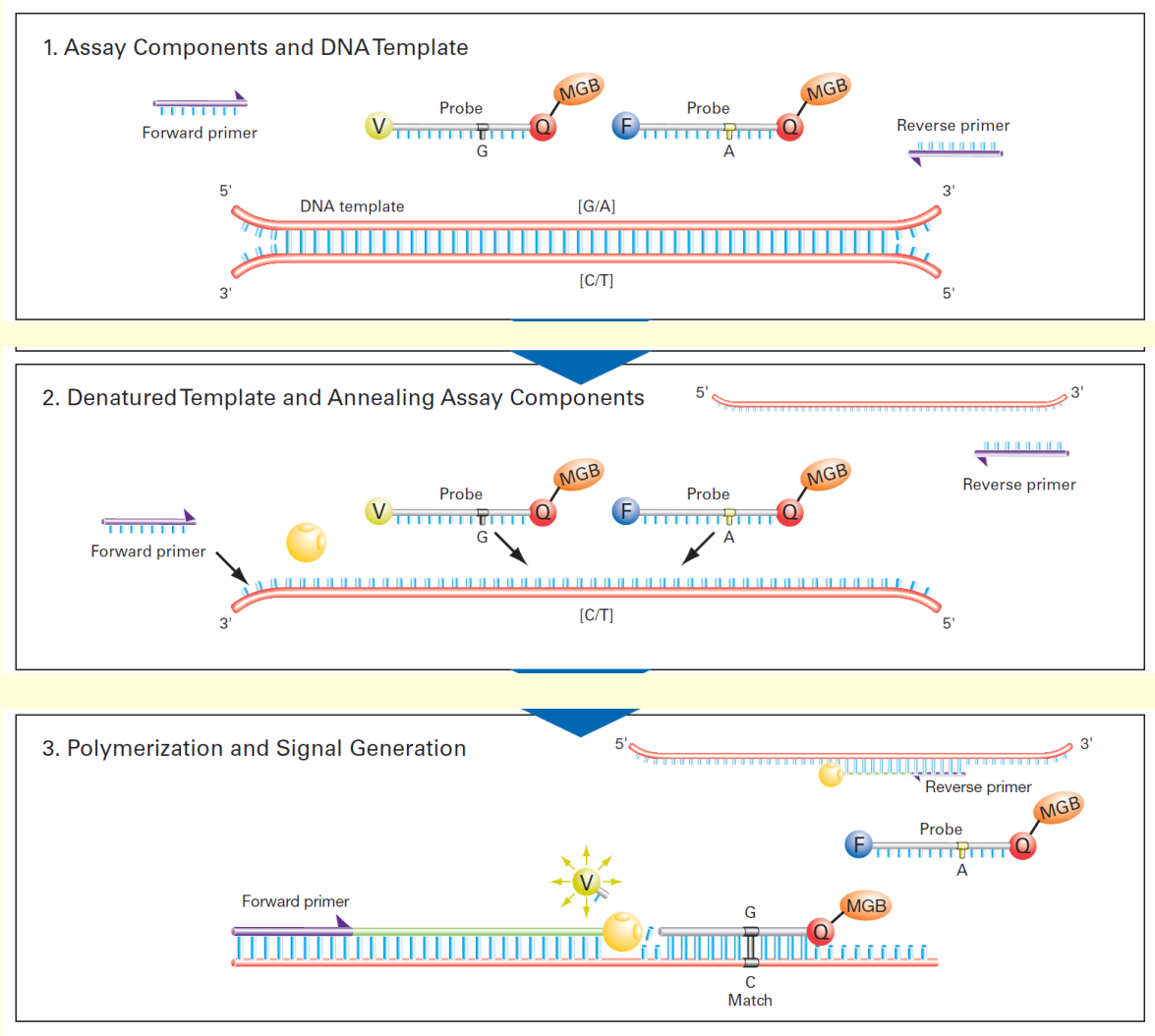
Limited material

FNA mediastinal lymphnode





Allele specific taqman probes = FAST



Rapid Mutation Analysis of Fine Needle Aspirates using allele-specific qPCR

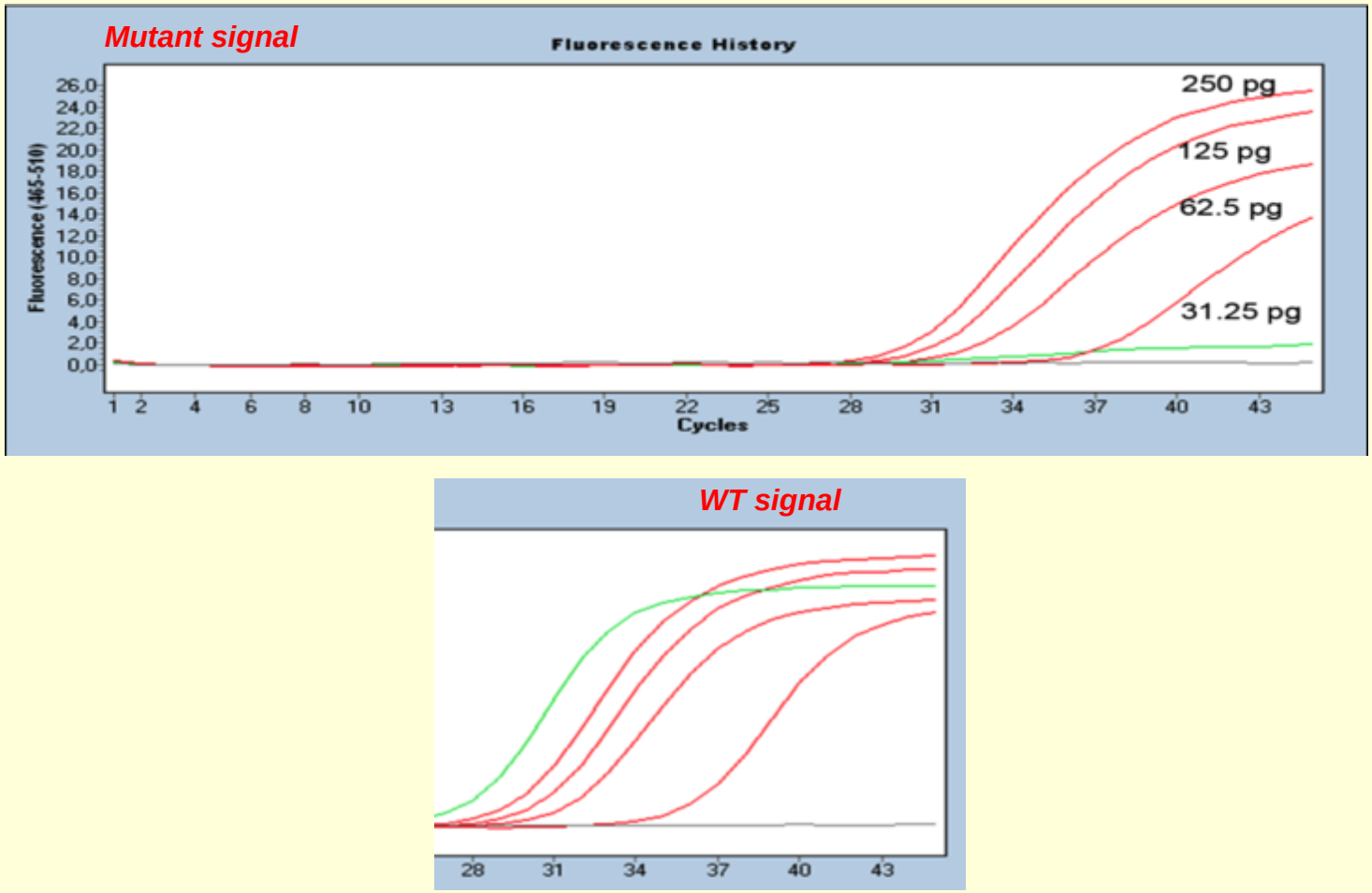
- EGFR: p.L858R*, exon 19 deletions*
- KRAS: p.G12S, p.G12R, p.G12C, p.G12D, p.G12A, p.G12V, p.G13D

- PIK3CA: p.E542K, p.E545K, p.H1047R
- BRAF: p.V600E, (p.V600K)
- NRAS, HRAS, KIT, IDH1/2, ….

R. van Eijk et al PlosONE 2011: 6 (3) e17791

And it works on minimal input DNA

Effect of the DNA concentration on the c.34G.T KRAS assay.



R. van Eijk et al PlosONE 2011: 6 (3) e17791

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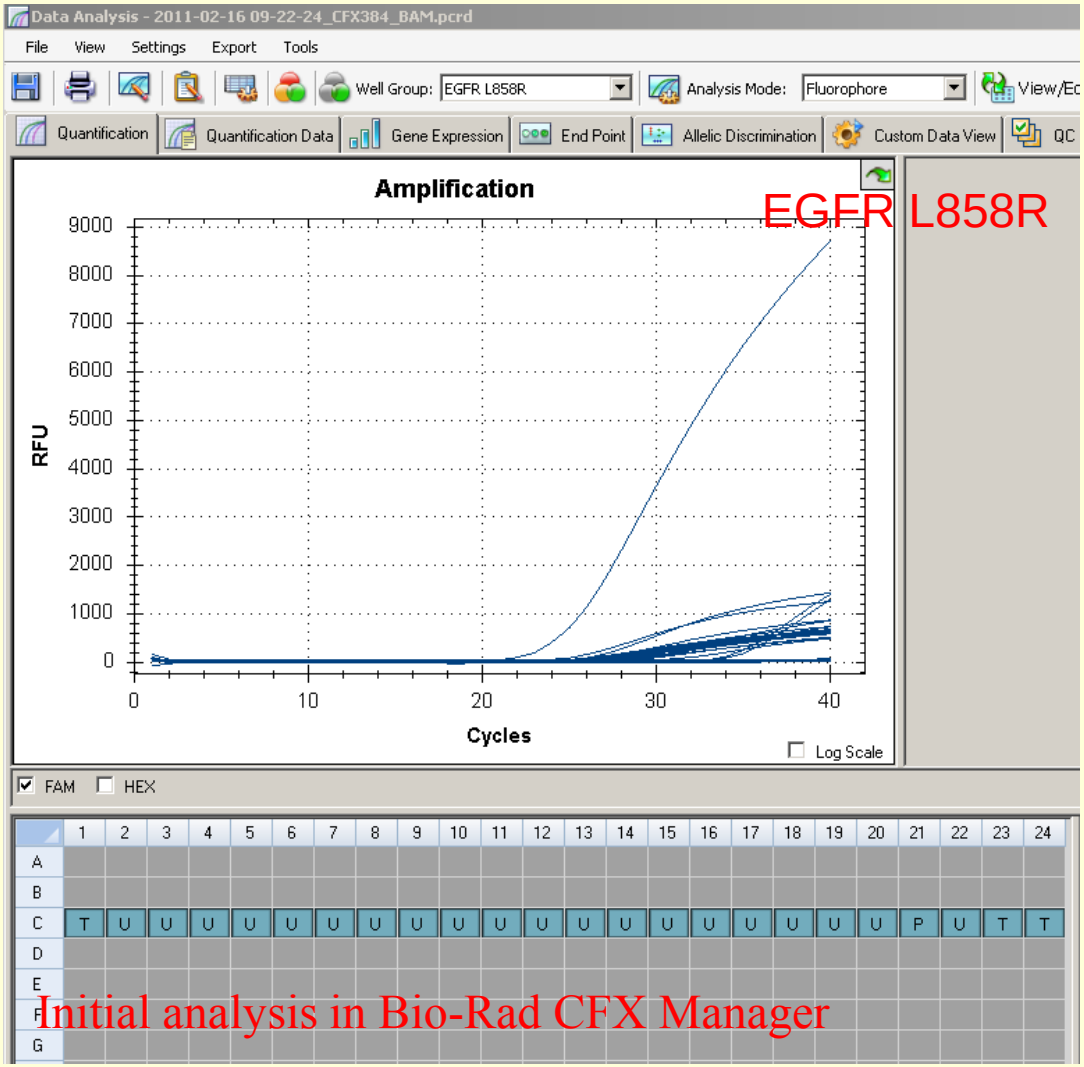
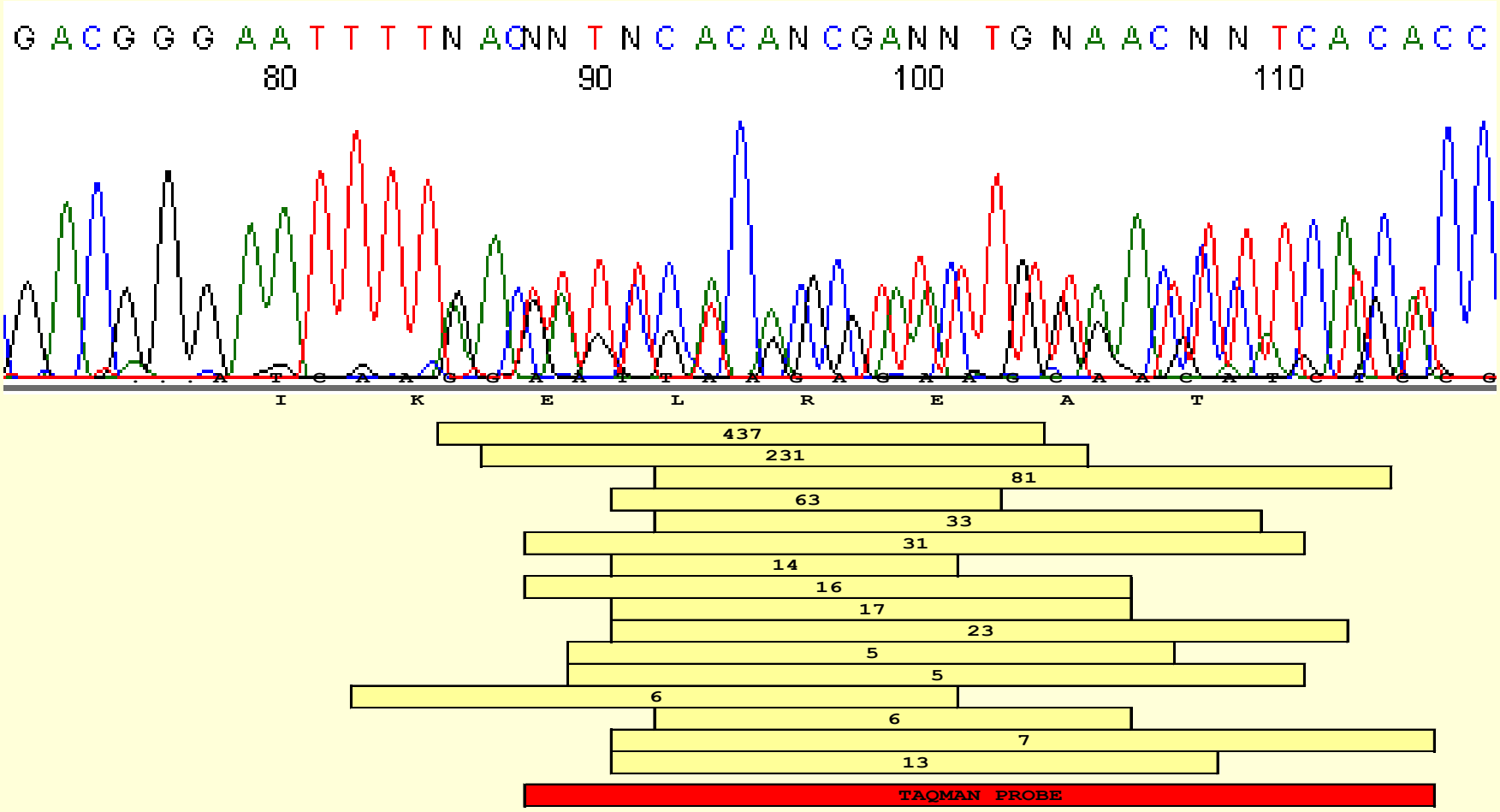


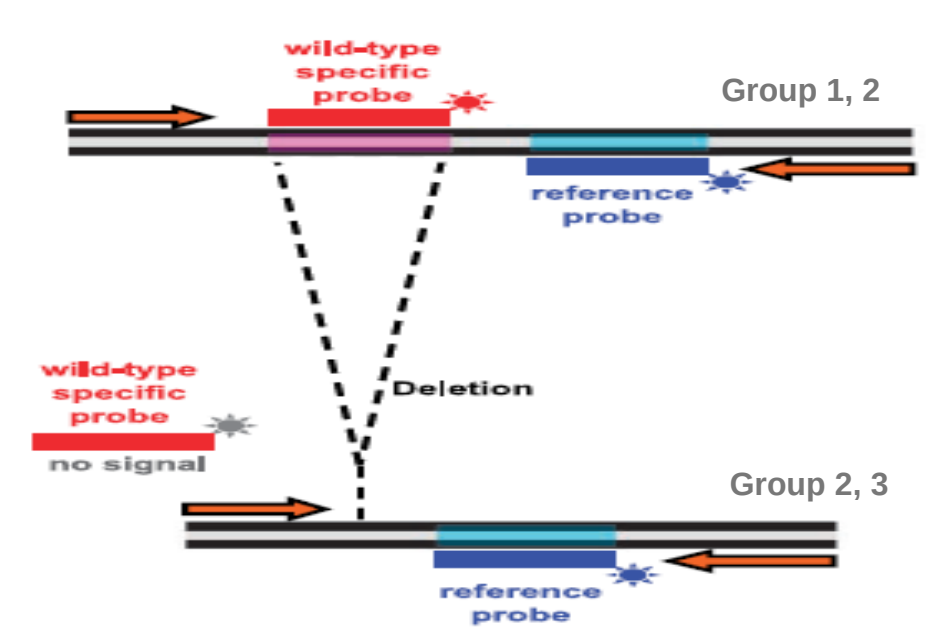
EGFR deletion exon 19

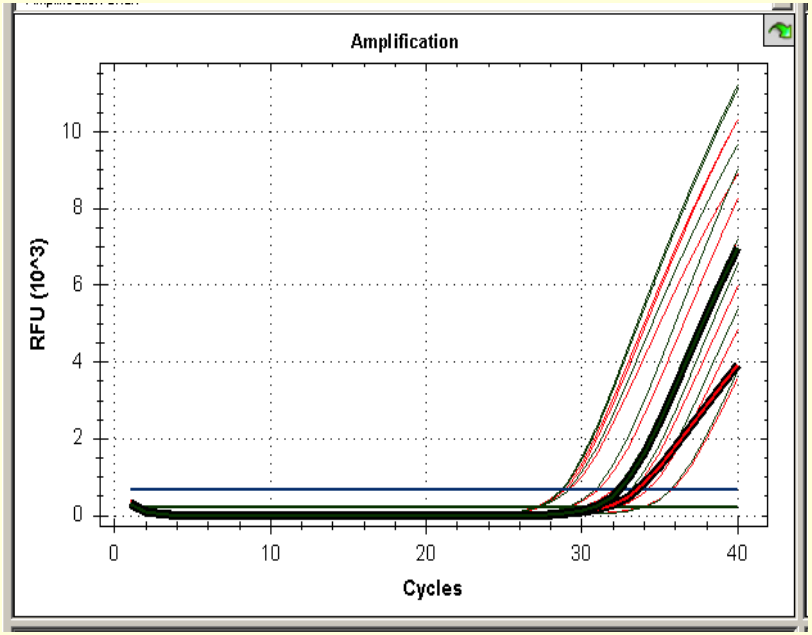
- ‘Classical detection’



FAST assay for exon 19 deletions

Wild type signal is lost if a deletion is present





Single-Molecule Detection of Epidermal Growth Factor Receptor Mutations in Plasma by Microfluidics Digital PCR in Non – Small Cell Lung Cancer Patients

Tony K.F. Yung,[1,2] K.C. Allen Chan,[1,2] Tony S.K. Mok,[1,3] Joanna Tong,[4] Ka-Fai To,[4] and Y.M. Dennis Lo[1,2,5]

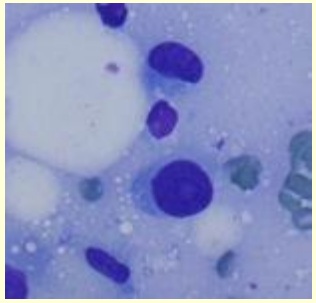
Clin Cancer Res 2009;15:2076-2084. Published online March 10, 2009.

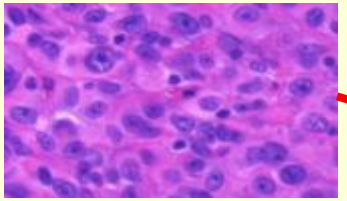
Rapid *KRAS*, *EGFR*, *BRAF* and *PIK3CA* Mutation Analysis of Fine Needle Aspirates from Non-Small-Cell Lung Cancer Using Allele-Specific qPCR

PLoS ONE | www.plosone.org March 2011 | Volume 6 | Issue 3 | e17791

Tumor heterogeneity in melanoma

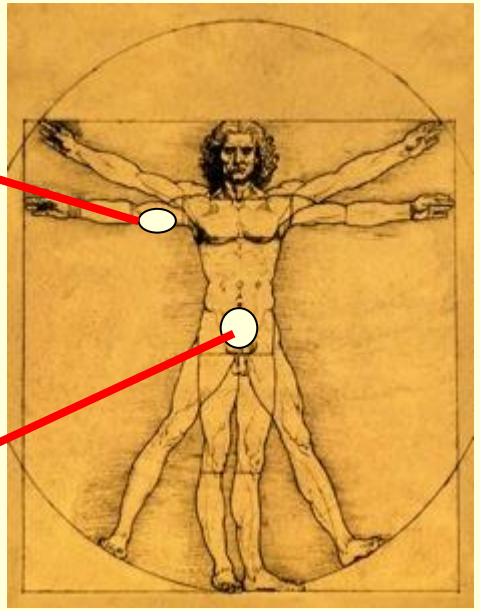
BRAFV600E



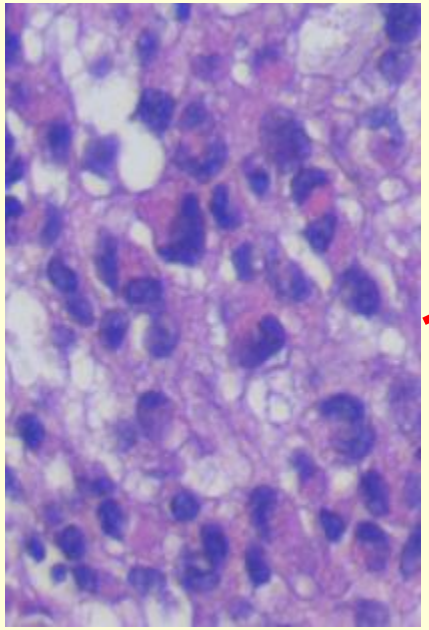


COBAS: BRAFV600 +

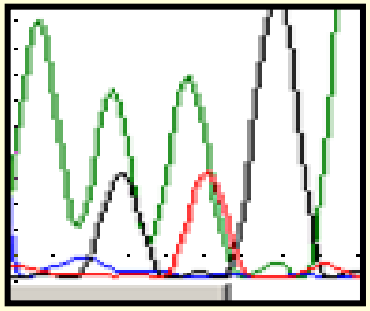
2 Subcutane lesions



NO BRAF V600E



GTG -> AAG: V600K



OPEN ACCESS Freely available online

PLoS one

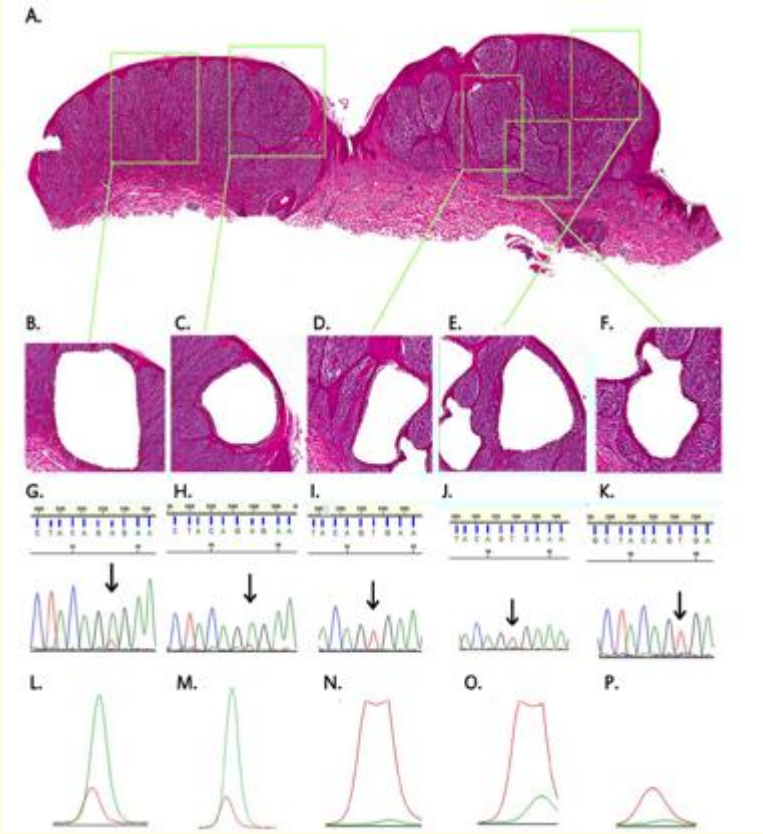
Intra- and Inter-Tumor Heterogeneity of *BRAF*^{V600E} Mutations in Primary and Metastatic Melanoma

Molly Yancovitz[1☯], Adam Litterman[1☯], Joanne Yoon[1], Elise Ng[1], Richard L. Shapiro[2], Russell S. Berman[2], Anna C. Pavlick[1,3], Farbod Darvishian[4], Paul Christos[5], Madhu Mazumdar[5], Iman Osman[1,3], David Polsky[1,4]*

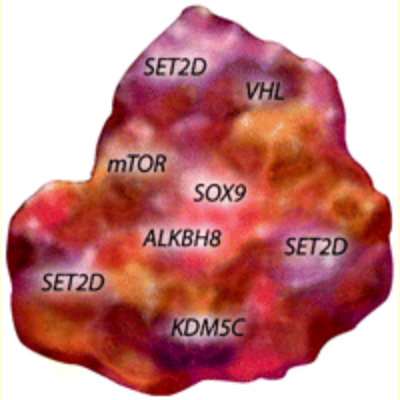
Table 4. BRAF mutation concordance between primary and metastatic specimens using MS-PCR.

Patient	Primary tumor	Metastatic tumor
1	Wild Type	Mutant
2	Wild Type	Mutant
3	Wild Type	Mutant
4	Wild Type	Mutant
5	Wild Type	Mutant
6	Wild Type	Mutant
7	Mutant	Mutant
8	Mutant	Mutant
9	Mutant	Mutant
10	Mutant	Mutant
11	Mutant	Mutant
12	Mutant	Mutant
13	Mutant	Mutant
14	Mutant	Mutant
15	Mutant	Mutant
16	Mutant	Mutant
17	Mutant	Wild Type
18	Mutant	Wild Type

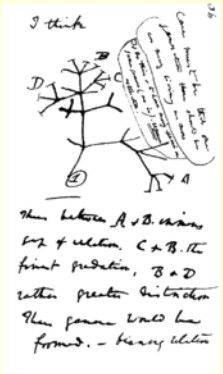
doi:10.1371/journal.pone.0029336.t004

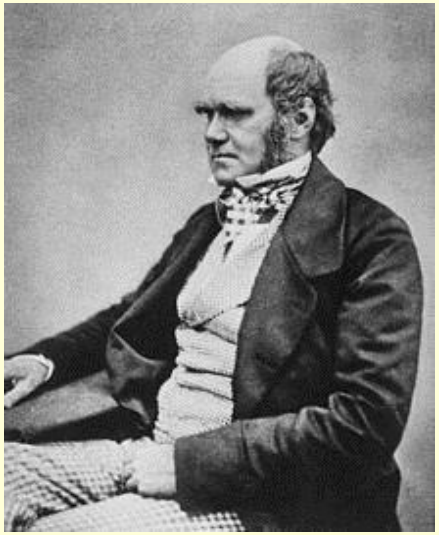


Tumor heterogeneity 1

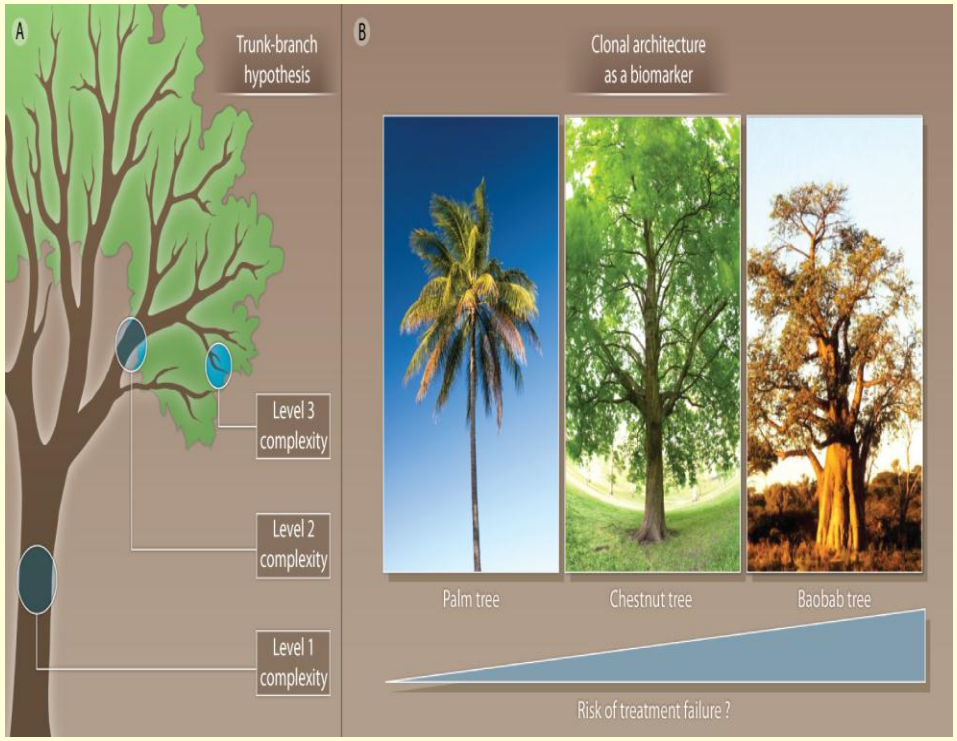


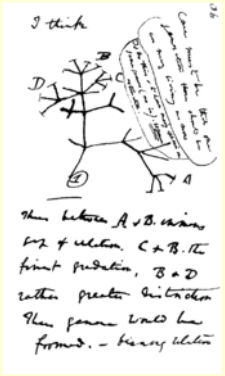
- Gerlinger M et al. N Engl J Med. 2012 (10):883-92. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.
- Cancer Discov. 2012; 2(8):660. A deeper look at tumor heterogeneity.

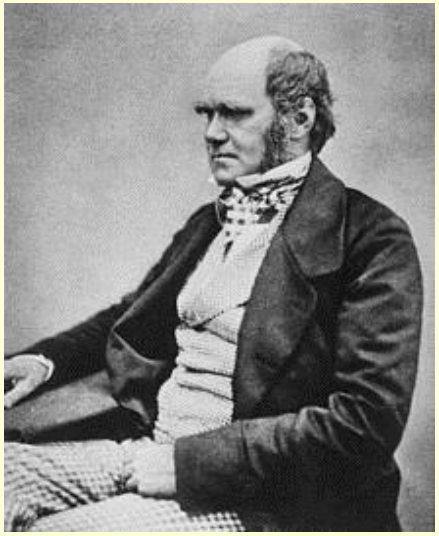




Tumor heterogeneity 2: **A trunk-branch model of intratumor heterogeneity.**







Yap T A et al. Sci Transl Med 2012;4:127ps10-127ps10

Charles Darwin: The origin of species

Published by AAAS

NGS current
vs
NGS limited material

- FFPE biopsies /FNA
- Mutoom Genomic, Methylomic, Transcriptomic, miRNA, fusion info needed.
- No DNA/cDNA amplification possible because of false positivity

NGS first/2nd/third

- FFPE Sequenom
- FFPE Target enrichment of selected gene sets

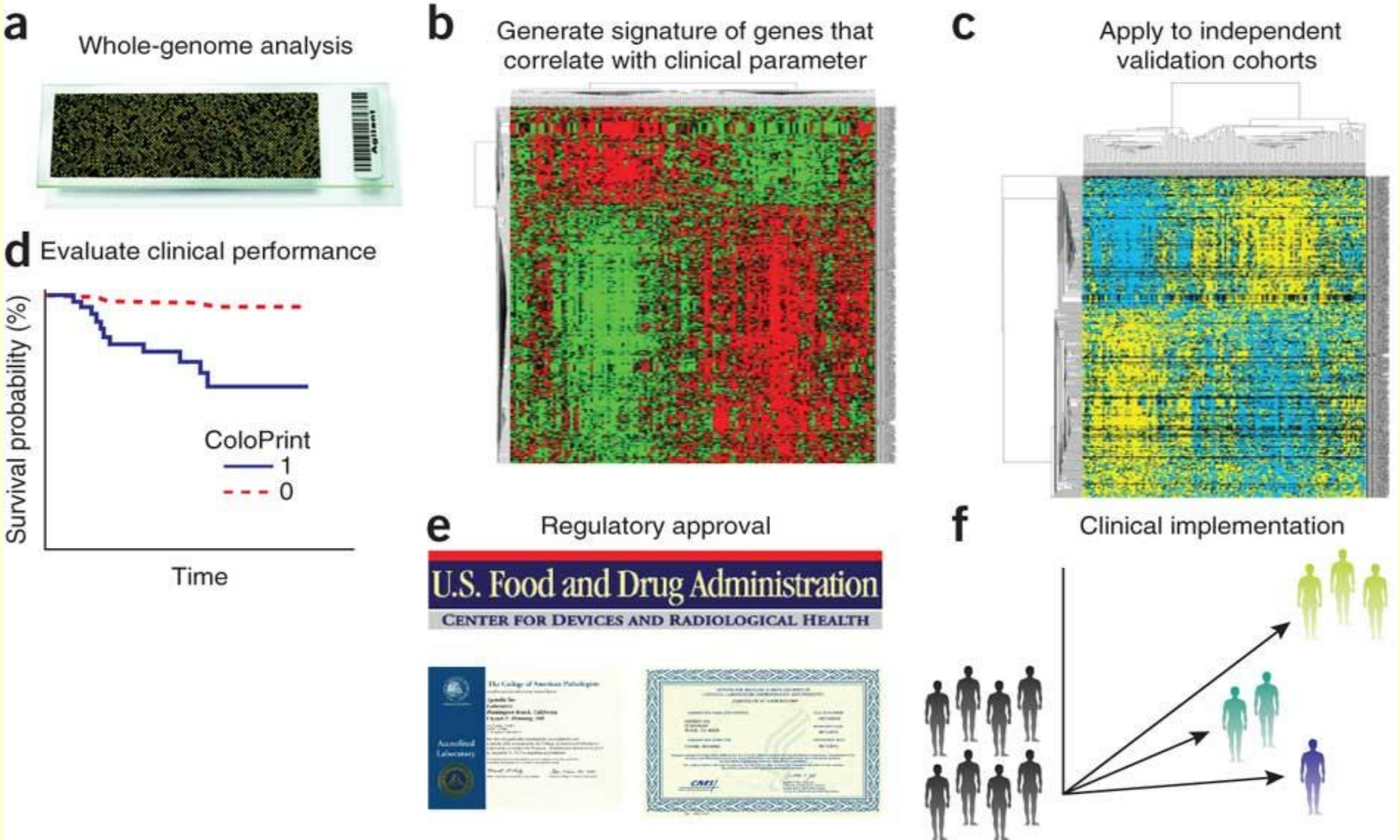
- Helicos
- Illumina high seq/my seq
- Ion/Proton Torrent
- Nanopore
- Complete genomics

Challenges:

- Frozen tissue vs FFPE
- False positivity rates? Bioinformatics
- Logistics?

Taming the dragon: genomic biomarkers to individualize the treatment of cancer

Nature Medicine 2011



Concluding remarks:

Improving the human condition with genomic medicine

http://personalgenome.com/

Read Vogelstein and Kinzler:

Winning the war: Science Parkour

www.ScienceTranslationalMedicine.org march 28 2012

Vogelstein and Kinzler:

Winning the war: Science Parkour

www.ScienceTranslationalMedicine.org 2012

- There is a difference between *proclaiming* a war and *winning* a war. In 1971, U.S. President Richard Nixon proclaimed a war on cancer
- Prevention and early detection
- Immunotherapy, targeted therapy, achieving victory.

- Hanahan and Weinberg, 2011